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Article

Development and characterization of pullulan-polymethacrylate free films as potential material for enteric drug release

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Free films of pullulan-polymethacrylate associations were produced by casting process to develop a novel target-specific material. For characterization, tests of water vapor permeability, swelling index, infrared absorption spectroscopy, thermogravimetric analysis, scanning electron microscopy and mechanical analysis were performed. The polysaccharide concentration directly influenced vapor permeability and swelling, increasing the values of the latter up to five times when added in a proportion of 20% (per weight). The individual properties of each polymer were maintained, and chemical interactions were not detected. The films were found to be thermally stable and they had unaltered mechanical properties with the addition of the polysaccharide. The microscopic analysis revealed rugosity that was proportional to pullulan and disorganization of the polymer network at pH 6.8. These results suggest that this novel material has potential for enteric drug release because of synergism between pH and enzyme dependence.

Keywords: Films. Polymeric material. Modified release/enteric release/oral delivery/controlled release. Glucans. Eudragit. Physicochemical characterization.

INTRODUCTION

Despite patients' acceptance and flexibility with regard to the concept of different methods of dosing, oral drug administration has some therapeutic limitations. Consequently, new formulations, such as modified release, have been proposed (Wasnik, Parmar, 2011). Release in distal regions of the gastrointestinal tract (GIT) is beneficial for the treatment of pathologies that affect the large intestine because the drug is released at the site of action, thus increasing the concentration of the drug at these regions, decreasing the ingested dose, and decreasing side effects (Freire *et al.*, 2006; Nadler, Kam, Rubinstein, 2010; Wasnik, Parmar, 2011; Shukla, Tiwari, 2012). Additionally, these target-specific systems are potentially adequate for the release of protein and peptide therapeutics and protection of their activity from hostile physiological conditions related to stomach pH and digestive enzymes (Freire *et al.*, 2006; Pinto, 2010; Wen, Park, 2010; Wasnik, Parmar, 2011).

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The polymethacrylate Eudragit® FS 30 D (EFS) is an aqueous dispersion at 30%, consisting of a copolymer of methylacrylate, methylmethacrylate, and methacrylic acid and possessing carboxylic acid as the functional group (Evonik, 2016). The dissolution of this synthetic polymer occurs via salt formation and water uptake in fluids at pH >7.0, which allows pH-dependent drug release (Dulin, 2010; Evonik, 2016). Therefore, EFS, when used as a coating in reservoir or in matrix systems, passes through the stomach and small intestine intact and is dissolved only when it contacts neutral-to-alkaline pH in the colonic segment (Ibekwe *et al.*, 2006).

However, these systems do not always accomplish the goal of efficacious drug release at the desired site. Some pathologies cause colon pH to undergo extreme variations (e.g., in inflammatory illness, such as ulcerative colitis and Crohn's disease) when the pH can change in the range of 4.7 to 2.3 (Freire *et al.*, 2006; Nadler, Kam, Rubinstein, 2010; Rabito *et al.*, 2012). This reduction is attributable to fermentation processes in the region (Nadler, Kam, Rubinstein, 2010). Moreover, coatings for colonic release that are exclusively sensitive to pH can present low specificity because they depend on other factors, such as intestinal transit and coating thickness, for drug release.

Therefore, the drug is at risk of being prematurely released or causing undesired and slow availability in the descending colon (Nadler, Kam, Rubinstein, 2010; Wen, Park, 2010).

One way to overcome these issues of pH-dependent release systems is the association of the physiological mechanisms present in the GIT. One can combine strategies of applying materials destined for pH-related release with materials that are sensitive to microbiota biodegradation. Thus, upon reaching the colon, both strategies can act synergistically, leading to disintegration of the pharmaceutical form and working as a safety device with target-specific capacity (Ibekwe *et al.*, 2008; Mcconnell, Short, Basit, 2008; Villanova, Oréfice, Cunha, 2010; Basit, Mcconnell, 2011).

Pullulan (PU) is a natural polymer that is extracellularly produced by the fungus *Aureobasidium pullulans*, in starch and sugar cultures; therefore, it is considered an exopolysaccharide (Xiao, Lim, Tong, 2012). It is characterized as a α-glucan and has a linear primary structure composed of subunits of maltotriose linked by α-1,6-glycosidic linkages (Pradella, 2006; Introzzi *et al.*, 2012). This structure allows PU to have good solubility in water (Xiao, Lim, Tong, 2012).

This exopolysaccharide has no odor, taste, or toxicity. The molecular weight is between 8000 and 2 000 000 Da, depending on the culture conditions and commercial application of PU (e.g., industrial, medical, and alimentary) (Pradella, 2006). Moreover, digestive enzymes in the human intestine do not degrade PU (Prajapati, Jani, Khanda, 2013). However, it promotes the growth of intestinal *Bifidobacterium spp* and it can be considered a prebiotic (Silva *et al.*, 2006). It can be used for modified drug release by acting as an enzyme-dependent polymer because it acts as a substrate for this ecosystem.

PU still presents adhesive properties and the capacity to form biodegradable and edible fibers and films (Cheng, Demirci, Catchmark, 2011). The films obtained from this exopolysaccharide are thermally stable, have elastic and antistatic properties, can be used for printing, and are directly compressible under heat and humidity. Additional characteristics include transparency, solubility in water, and insolubility in organic solvents. Furthermore, because of its filmogenic particularities, the addition of a plasticizer is not necessary (Singh, Saini, Kennedy, 2008; Islam, Yeum, Das, 2012; Wu *et al.*, 2013).

To discover a convenient way to explore pH and enzyme-dependent synergistic properties, the goal of the present study was to develop a novel polymeric material destined for drug release in the distal part of the GIT by associating the polymethacrylate EFS and polysaccharide PU.

MATERIAL AND METHODS

Material

Alimentary-grade pullulan (CAS 9057-02-7; Tovani Benzaquem Ingredientes®, Brazil), Eudragit® FS 30 D (CAS 26936-24-3; Evonik®, Germany), polysorbate 80, glyceryl monostearate, sodium chloride, hydrochloric acid, sodium hydroxide, monobasic potassium phosphate, and distilled water were used. All of the reagents had analytical purity.

Preparation of free films

The films were produced using a casting process, in which the aqueous dispersion of the synthetic polymer EFS was associated with the polysaccharide PU in varying mass proportions: 100:00, 95:05, 90:10, and 80:20 respectively.

Initially, the polymethacrylate EFS was shaken at 50 ºC for 30 min with a 5% emulsion of the sliding glyceryl monostearate and wetting agent polysorbate 80 (2%) according to the methods proposed by Rabito *et al.* (2012) to obtain a homogeneous dispersion. Concomitantly, PU was dissolved in distilled water, heated at 50 ºC, and slowly added to the polymethacrylate dispersion. The final dispersion after total homogeneity was poured into Teflon molds in 10 ml samples and maintained in an oven at 50 ± 2 ºC for approximately 24 h. After complete solvent evaporation, the films were carefully removed from the molds and stored in desiccators that contained silica gel. For infrared testing, thermal behavior analysis, and mechanical analysis, films of 100% PU were also prepared. The final polymeric concentration was maintained at a constant 4% w/v for all of the formulations.

The film thickness was determined using a Mitutoyo® micrometer with 0.01 mm precision. Five different points were measured in three samples of isolated films for each association. Statistical analysis was performed by means of Analysis of Variance using a confidence level of 95%.

Water vapor transmission (WVT)

Method "B" (E 96-66) of the American Society for Testing and Materials (ASTM, 1966) was used to verify the films' water vapor permeability. Films with an area of 10 cm² were fixed in Payne permeability cups (Braive Instruments) that contained 10 ml of distilled water, and the set was weighed at predetermined times (0, 24, 48, 72, 96, and 120 h) until obtaining constant rate.

Tests were performed in triplicate, and the cups were stored in desiccators that contained dehydrated silica gel,

which was replaced at each weighing. From the mass loss, the rate of water vapor transmission was calculated using Equation 1 (Van Den Mooter, Samyn, Kinget, 1994):

$$WVT = g \times 24 / t \times a \qquad \text{(Equation 1)}$$

where *g* is the mass loss (g), *t* is the time in hours during which the weights were monitored (h), and *a* is the film area (m^2).

Swelling index (Is%)

To evaluate the swelling index of the polymer materials, film samples were sliced with surgical scissors at approximately 1 cm^2. To remove any residual humidity, the samples were left in an oven at 50 ºC until reaching a constant weight.

Each sample was weighed on an analytical scale to obtain the dry weight and immersed in simulated gastric fluid (SGF; pH 1.2) or simulated intestinal fluid (SIF; pH 6.8) prepared according to the 35th edition of the United States Pharmacopeia (USP, 2011), but without the addition of enzyme. At each interval (10, 30, 60, 90, 120, 150, and 180 min) (Ghaffari *et al.*, 2007), the films were carefully removed from the immersion media, and the excess liquid was removed with filter paper. The samples were weighed again to determine hydration. The procedure was performed in triplicate.

The films' swelling indices (Is%) were established according to Equation 2, where *Wd* represents the film's dry mass (g), and *Ws* represents the film's mass after immersion (g).

$$Is\% = (Ws - Wd) \times 100 / Wd \qquad \text{(Equation 2)}$$

Fourier transform infrared spectroscopy (FTIR)

The infrared absorption spectroscopy assay was performed using the Attenuated Total Reflectance technique with 32 scans at a pressure of 530 pounds per square inch (psi) using an FTIR BOMEM–MB–100 (Michelson®) spectrophotometer.

Thermogravimetric analysis (TGA)

The thermal stability of the different compositions of polymeric films was evaluated using thermogravimetric analysis with a Simultaneous Thermal Analyzer STA 409 PC Luxx (Netzsch®). Approximately 6 mg samples were previously dehydrated, entrapped in a Pt-Rd crucible, and subjected to an atmosphere with a 30 mL/min flow of nitrogen gas and increasing temperature of 22-900 °C at 10 °C/min.

Characterization by Scanning electron microscopy

Morphological characterization was performed using scanning electron microscopy (SEM). The films were analyzed while drying and after swelling for 3 h in SGF (pH 1.2) and SIF (pH 6.8). After hydration, the pellicles were frozen at -18 °C to conserve their structures and then lyophilized at -55 °C for 6 h in a freeze drying Liotop® L101 (Liobras®). The samples were metallized with gold and surface micrographs were obtained with a Scanning Electron Microscope SS-550 SUPERSCAN (Shimadzu®) operated at 10 keV.

Mechanical properties

According to the ASTM D882 method, mechanical analyses were performed with a texturometer TA.XT2 (Stable Microsystems®) equipped with a 5 kg load cell in an environment with controlled temperature (25 ± 1 °C) and humidity (55 ± 3%).

The samples were cut into sizes of 50 mm × 15 mm and adjusted to the equipment grips (Fajardo *et al.*, 2012). The parameters were the following: tension of rupture, maximum elongation, and Young's modulus, directly determined by Exponent 6.1.1.0 software (Stable Microsystems®). Four samples of each association were analyzed, and the averages were calculated.

Statistical analysis

Analysis of variance for multiple comparisons were used to compare the values obtained with different proportions of EFS and PU. (Villanova, Oréfice, Cunha, 2010; Xiao, Lim, Tong, 2012). Tukey's test was used for *posthoc* comparisons of the different polymer associations (Bunhak *et al.*, 2007; Rabito *et al.*, 2012). Student's t-test was used in the swelling index to compare the hydration between two fluids. The level of statistical significance was set at $p < 0.05$

RESULTS AND DISCUSSION

Morphological-macroscopic evaluation and film thickness

Films that had air bubbles, cracks, or deformities were discarded to avoid interference with the analysis.

TABLE I - Values of mass loss and water vapor transmission (n=3). Means in a column with different superscripts (a - c) are significantly different ($p<0.05$, by Tukey's test)

Polymer association	WVT (g/m^2 24h)	Values of mass loss (g/120h)
EFS 100:00 PU	170.69 ± 20.55^a	0.85 ± 0.10
EFS 95:05 PU	234.65 ± 23.56^b	1.17 ± 0.12
EFS 90:10 PU	263.19 ± 18.72^b	1.32 ± 0.09
EFS 80:20 PU	550.67 ± 11.64^c	2.75 ± 0.06

With regard to opacity, transparency reduced as the polysaccharide concentration increased. The mean thickness was 0.11 ± 0.01 mm, and no significant differences were found among the samples that had different concentrations of synthetic and natural polymers ($p>0.05$).

Water vapor transmission

Table I shows that water vapor permeability was proportional to PU concentration ($p < 0.05$). Cavalcanti *et al.* (2002) and Bunhak *et al.* (2007) also observed an increase in WVT when working with combinations of polymethacrylate and polysaccharides, like galactomannans and chondroitin sulfate respectively. This is mainly attributable to an increase in the system's hydrophilicity produced by the natural polymer. However, the WVT obtained in the EFS:PU formulations were inferior to those resulting in the cited works, which represents an advantage, since there is a greater prevention of the premature release of drugs in the upper parts of the GIT.

Additionally, Figure 1 shows a linear relationship between the mass loss and time. Akhgari *et al.* (2006) obtained similar results working with EFS and inulin.

Swelling index

Figure 2 shows the swelling index obtained after 180 min immersion in SGF and SIF. Statistical analysis was realized for the swelling index obtained in all the tested times of immersion (10, 30, 60, 90, 120, 150 and 180 min), and, after film immersion in SGF, significant differences ($p<0.05$) occurred in the samples with 100:00 and 90:10 proportions and 100:00 and 80:20 proportions. For the films immersed in SIF, significant differences were found between films with 100:00 and 80:20 proportions and 95:5 and 80:20 proportions. Because of the high hydrophilicity of the natural polymer, films with higher concentrations of PU presented higher levels of hydration.

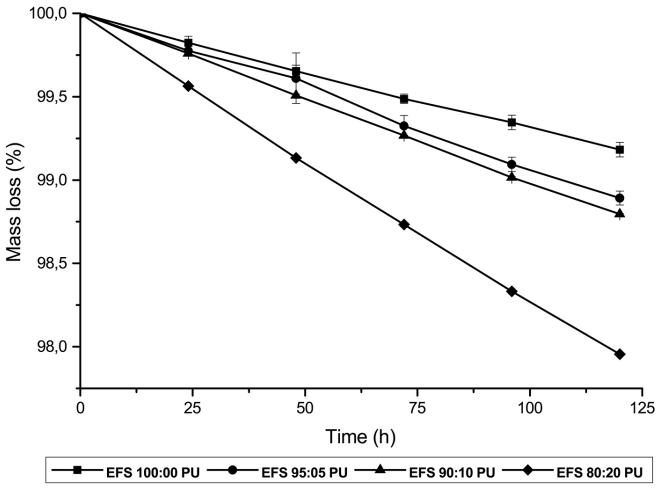

FIGURE 1 – Mass loss for films prepared at different EFS:PU ratios (n=3).

Notable are the results obtained with samples of free films with different associations immersed in pH 6.8 ($p<0.05$; i.e., higher Is%). These findings suggest a predominance of EFS properties because they are pH-dependent and soluble in neutral and slightly alkaline pH. In SIF, this polymer naturally ionized, absorbing large amounts of solvent. Akhgari *et al.* (2006) previously reported this phenomenon and attributed it to the characteristics of the base synthetic polymer.

Akhgari *et al.* (2006) working with various combinations of EFS and inulin, obtained Is% similar to those obtained for the EFS:PU formulations. However, as for the WVT assay, the swelling values in SGF found in the present study were lower, demonstrating a greater resistance to premature drug release in the upper regions of the GIT.

Infrared absorption spectroscopy

Figure 3 shows that both EFS and PU presented bands in regions similar to the ones reported in the literature. The film that contained only EFS displayed a spectrum with bands at 1161 cm^{-1} and 1194 cm^{-1} due to

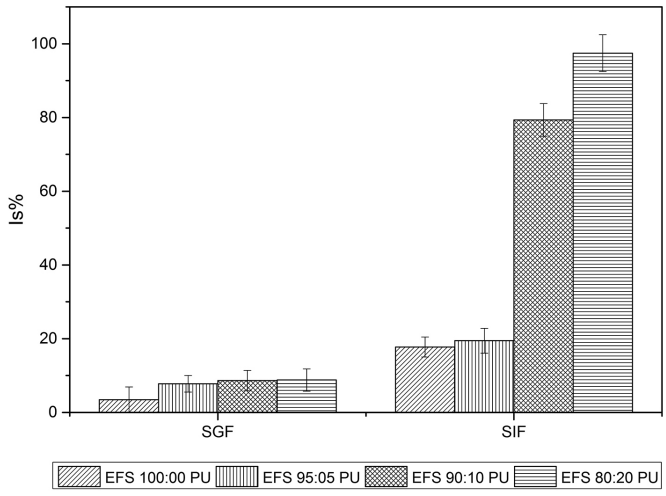

FIGURE 2 - Swelling index of films after 180 min immersion in SGF (pH 1.2) and SIF (pH 6.8) (n=3).

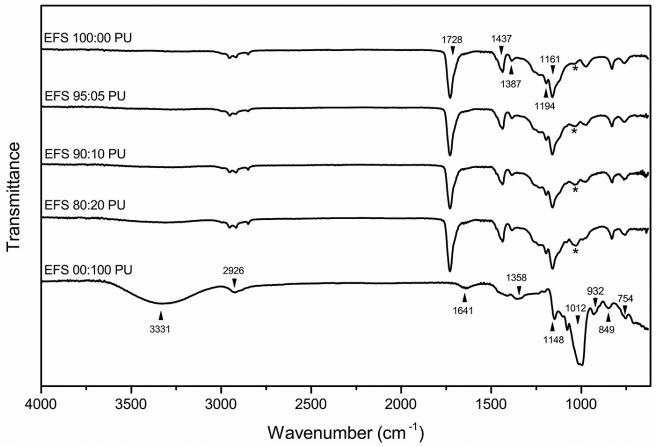

FIGURE 3 - FTIR of polymethacrylate films that contained 0-20% PU and pure PU.

ester vibrations, a band at 1728 cm^{-1} that corresponded to vibrations of C=O of esterified carboxyl groups, and bands at 2900-3000 cm^{-1}, 1387 cm^{-1}, and 1437 cm^{-1} due to vibrations of CH_x (Evonik, 2016; Moustafine *et al.*, 2012).

The spectroscopy of PU showed bands at 3331 cm^{-1} that corresponded to repeated units of hydroxyl groups, 2926 cm^{-1} due to vibrations of C-H linkages, 1641 cm^{-1} caused by C-O-C linkages, 1358 cm^{-1} caused by C-O-H linkages, 1148 cm^{-1} caused by C-O-C linkages, 1012 cm^{-1} caused by C-O linkages, 754 cm^{-1} that demonstrates the linkages predominant in PU between glucose units α-(1,4) and α-(1,6), 932 cm^{-1} related to the presence of glycosidic linkages α-(1,6), and 849 cm^{-1} due to α-configuration (Shingel, 2002; Gniewosz, Duszkiewicz-Reinhard, 2008; Singh, Saini, 2008; Karim *et al.*, 2009; Cheng, Demirci, Catchmark, 2010; Constantin *et al.*, 2011; Bhat *et al.*, 2012; López-Rubio *et al.*, 2012; Sugumaran *et al.*, 2013).

In the compositions 95:5, 90:10, and 80:20, we observed similarities with the spectra of pure EFS that were attributable to the high concentration of this base polymer. For these same associations, in the 1039 cm^{-1} spectrum region, an increase in the band intensity it was noticed that was proportional to the addition of the polysaccharide, which is likely related to axial symmetric bending of the PU's C-O groups.

Because of the lack of a shift or appearance of new bands, we conclude that only a physical interaction exists between the polymers, without the presence of intermolecular interactions.

Thermogravimetric analysis

The results of the TGA are presented in Figure 4. The degradation temperatures of EFS and PU were close to 400 ºC and 300 ºC, respectively. With regard to the associated films, the addition of PU up to 20% caused the presence of two distinct degradation steps: one related to the polysaccharide and one related to polymethacrylate. This finding suggests a physical interaction between the chains of the synthetic and natural polymers. Additionally, PU presented higher losses of superficial humidity compared with the other samples because of its high hydrophilicity.

From the first derivative of the TGA (DTG; Figure 5), it was noticed that the temperature and speed of maximal mass losses for the natural polymer (319 °C) were lower than the sample that contained only EFS (410 °C). Karim *et al.* (2009), Constantin *et al.* (2011), and Teramoto and Shibata (2006) found similar degradation values of PU as the ones in the present study, thus confirming the correct identification of this polysaccharide.

These results suggest that the films based on EFS and PU present suitable thermal stability, when considering that the process of pellicle coating is performed at temperatures of about 40 ºC.

Microstructure characterization by Scanning electron microscopy

As shown in Figure 6, dry films showed minor changes in morphology. When they were immersed in pH 1.2, slight superficial disarrangement due to PU hydrophilicity was observed. At pH 6.8, this disarrangement was more evident, in which the films with 90:10 and 80:20 compositions presented a deeply disordered polymer network. This phenomenon may be related to the pH-dependent properties of the synthetic polymer and structural alterations of the PU network.

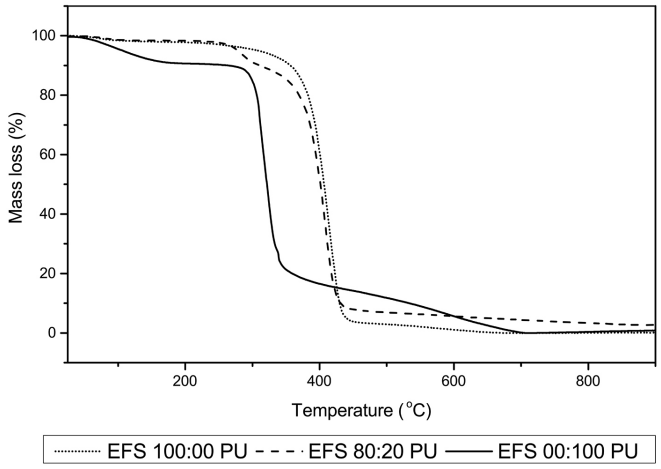

FIGURE 4 - Thermogravimetric analysis of samples of EFS 100:00 PU, EFS 80:20 PU, and EFS 00:100 PU.

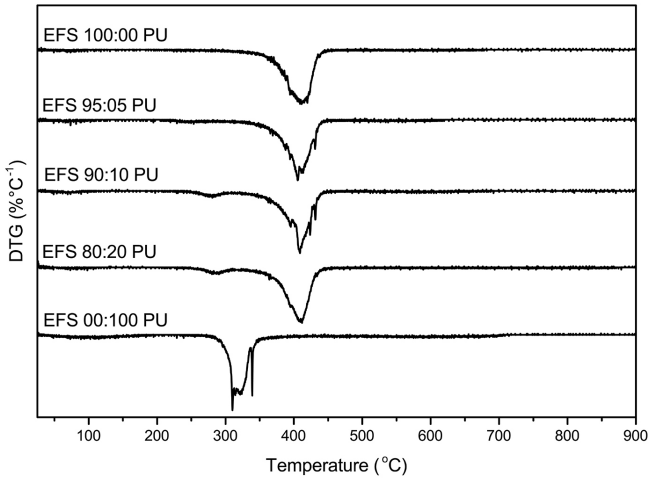

FIGURE 5 - First-derivative TGA of the films with various concentrations.

Mechanical properties

A film coating's mechanical stability is critical for avoiding breaks and providing adequate physical protection in pharmaceutical forms. The human organism provides several sources of stress for a drug that reaches the GIT, such as shear force that results from the motility of the upper region and hydrostatic pressure exerted by the system against the pellicle after contact with bodily fluids (Karrout *et al.*, 2009a; Karrout *et al.*, 2009b).

Table II shows that the film of pure polymethacrylate and samples that contained both polymers did not differ in terms of the analyzed parameters in the tensile assay (p>0.05). Therefore, the addition of the polysaccharide did not alter the mechanical characteristics of EFS, in which the properties were adequate for coating

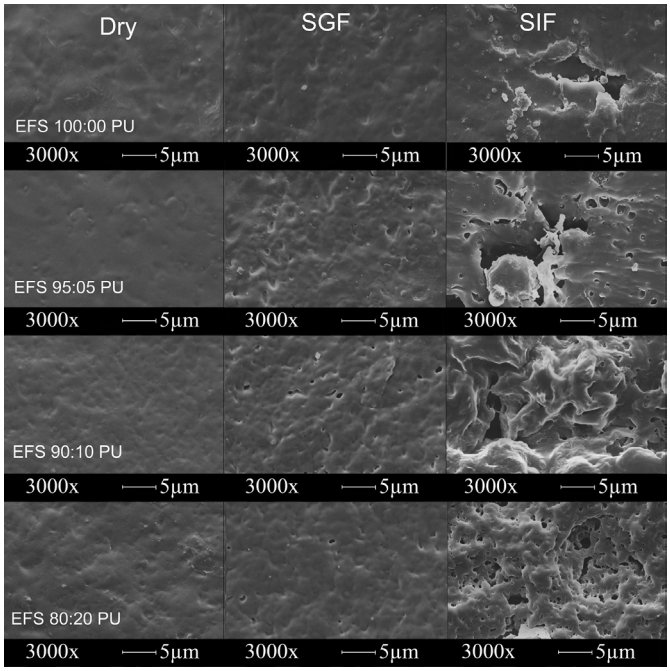

FIGURE 6 - SEM of dry EFS:PU films and after immersion for 180 min at SGF and SIF (3000x magnification).

pharmaceutical forms, since it is a product marketed for this purpose.

Another important result was the reduction of Young's modulus values, characterizing a polymer material with a relative flexibility. Polymers used as pharmaceutical coatings must be elastic and flexible to be adaptable to the deformations and edges associated with pharmaceutical forms without rupturing (Murthy Dwibhashyam, Ratna, 2008).

On the other hand, the film that contained only PU showed superior values for all parameters. Because it has high hydrophilicity, this polymer presents high amounts of intermolecular hydrogen linkages between hydroxyl groups, which can justify the higher thresholds of rupture tension (Tang *et al.*, 2010). Comparisons between different studies are difficult because pellicle preparation and methods influence the tensile test results (Macleod, Fell, Collett, 1997). However, Xiao, Lim and Tong (2012) and Teramoto and Shibata (2006) obtained maximal elongation results for PU that were similar to the present results.

CONCLUSION

The novel polymer material prepared by incorporating PU with the polymethacrylate EFS presented promising characteristics and properties for application in systems destined for enteric drug release. It is observed an adequate resistance to GIT fluids; and

TABLE II - Mechanical properties of EFS:PU films (n=4). Means in a column with different superscripts (a – b) are significantly different (p<0.05, by Tukey's test)

Sample	Rupture tension (KPa)	Maximal elongation (%)	Young's modulus (KPa)
EFS 100:00 PU	18.31 ± 2.66[a]	0.61 ± 0.13[a]	25.17 ± 2.39[a]
EFS 95:05 PU	17.10 ± 4.17[a]	0.71 ± 0.19[a]	24.50 ± 5.12[a]
EFS 90:10 PU	19.80 ± 7.58[a]	0.68 ± 0.41[a]	24.25 ± 1.60[a]
EFS 80:20 PU	15.31 ± 1.48[a]	1.17 ± 0.58[a]	22.78 ± 5.19[a]
EFS 00:100 PU	71.41 ± 5.96[b]	2.26 ± 0.82[b]	42.35 ± 5.74[b]

mechanical properties and thermal stability compatible with processes required for film coating. The individual properties of each polymer in the associations were preserved, causing only physical interactions between the content of the formulations. The preserved properties open up opportunities to exploit the synergism between the pH sensitivity properties of polymethacrylate and enzyme-dependent properties of PU. In addition, when compared with some other polymeric films developed with the purpose of enteric release (which use various methacrylates:polysaccharides formulations), proved to be more resistant to premature release of drugs in the upper parts of the GIT.

These findings suggest that the material obtained in the present study has properties that may be applied as coatings for colon-specificity. However, complementary in vivo and in vitro assays are needed to confirm this indication.

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